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ORIGINAL ARTICLE

Distinct "Immunoallertypes" of Disease and High Frequencies of Sensitization in Non–Cystic Fibrosis Bronchiectasis

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Abstract

Rationale: Allergic sensitization is associated with poor clinical outcomes in asthma, chronic obstructive pulmonary disease, and cystic fibrosis; however, its presence, frequency, and clinical significance in non–cystic fibrosis bronchiectasis remain unclear.

Objectives: To determine the frequency and geographic variability that exists in a sensitization pattern to common and specific allergens, including house dust mite and fungi, and to correlate such patterns to airway immune-inflammatory status and clinical outcomes in bronchiectasis.

Methods: Patients with bronchiectasis were recruited in Asia (Singapore and Malaysia) and the United Kingdom (Scotland) ($n = 238$), forming the Cohort of Asian and Matched European Bronchiectasis, which matched recruited patients on age, sex, and bronchiectasis severity. Specific IgE response against a range of common allergens was determined, combined with airway immune-inflammatory status and correlated to clinical outcomes. Clinically relevant patient clusters, based on sensitization pattern and airway immune profiles ("immunoallertypes"), were determined.

Measurements and Main Results: A high frequency of sensitization to multiple allergens was detected in bronchiectasis, exceeding that in a comparator cohort with allergic rhinitis ($n = 149$). Sensitization was associated with poor clinical outcomes, including decreased pulmonary function and more severe disease. "Sensitized bronchiectasis" was classified into two immunoallertypes: one fungal driven and proinflammatory, the other house dust mite driven and chemokine dominant, with the former demonstrating poorer clinical outcome.

Conclusions: Allergic sensitization occurs at high frequency in patients with bronchiectasis recruited from different global centers. Improving endophenotyping of sensitized bronchiectasis, a clinically significant state, and a "treatable trait" permits therapeutic intervention in appropriate patients, and may allow improved stratification in future bronchiectasis research and clinical trials.

Keywords: bronchiectasis; sensitization; allergy; house dust mite; *Aspergillus*

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At a Glance Commentary

Scientific Knowledge on the Subject: Atopy and sensitization are established prognostic indicators in chronic respiratory diseases, including asthma and chronic obstructive pulmonary disease, where they represent "treatable traits." Their role in bronchiectasis not due to cystic fibrosis is unclear, and few studies have assessed the frequency of atopy in adult bronchiectasis. Reports to date are limited to single center studies, relatively small cohorts, and have demonstrated conflicting results.

What This Study Adds to the Field: We report what is, to our knowledge, the largest multicenter study of atopy in bronchiectasis, including patients from geographically distinct Asian (Singapore and Malaysia) and European (Scotland) cohorts. Our results illustrate high rates of allergic sensitization against a broad panel of allergens, including house dust mite and fungi that correlate with worse clinical outcome. The airway inflammatory profile of sensitized bronchiectasis reveals novel endophenotypes of disease in our "matched" and geographically distinct populations. This work demonstrates the importance of identifying sensitization and atopy in bronchiectasis, its clinical relevance, and geographic variability. We further demonstrate two clinically relevant endophenotypes, each driven by a specific allergen response profile and airway immune signature: fungal driven proinflammatory and house dust mite driven chemokine dominant. These "immunoallertypes" are clinically relevant and identify "high risk" subgroups of bronchiectasis where appropriate therapeutic intervention may be offered.

Bronchiectasis not due to cystic fibrosis (CF) is a disease experiencing a clinical and research renaissance (1). Characterized by persistent cough, mucopurulent secretions, and recurrent infection, permanent and irreversible bronchial dilatation ensues (2). The failure of therapies used in CF to translate to bronchiectasis is exemplified by trials of recombinant DNase therapy, which, in contrast to CF, increases exacerbations in bronchiectasis (3). Such failures suggest fundamental differences in disease-associated mechanisms, necessitating data-driven, endophenotyping approaches toward improved patient stratification in this heterogeneous disease (2). Although disease-associated effects on host immunity, infection, and inflammation are recognized, the role of allergic sensitization in the setting of bronchiectasis lacks dedicated study.

Atopy is a known risk factor for the development and/or progression of chronic respiratory disease, including asthma, chronic obstructive pulmonary disease (COPD), and CF (4, 5). Although allergic bronchopulmonary aspergillosis (ABPA) is an identified cause for bronchiectasis, the specific role of atopy and sensitization as a consequence of disease remains to be established. This is particularly important, as the presence of atopy, fungal sensitization and ABPA in asthma, COPD, and CF are all recognized associations of poorer clinical outcome, including decreased pulmonary function, more frequent exacerbations, and even the development of bronchiectasis (4, 6).

Prior reports of atopy in bronchiectasis are small, conflicting, and limited to single centers. In addition, none have thus-far addressed the potential for geographic variation across countries with differing allergen exposures and climates.

Here, we describe the largest study of atopy in bronchiectasis to date, including patients from clinically "matched" cohorts of Asian and European origin. Sensitization to a range of allergens was assessed with their clinical associations and accompanying airway inflammatory signatures. This allows patient stratification into clinically relevant groups, defined as "immunoallertypes"; specific endophenotypes of "sensitized bronchiectasis" with therapeutic implications. Some of the results of these studies have been previously reported in the form of abstracts (7, 8).

Methods

Study Population(s)

Patients with stable bronchiectasis, defined by British Thoracic Society guidelines, were recruited across three countries as part of a study, the cross-sectional CAMEB (Cohort of Asian and Matched European Bronchiectasis) (9, 10). Recruitment included three sites in Singapore (Singapore General Hospital, Changi General Hospital and Tan Tock Seng Hospital, Singapore; $n = 124$), one Malaysian site (UKM Medical Centre, Kuala Lumpur, Malaysia; $n = 14$), and an age-, sex-, and disease severity–matched group from a single European site (Ninewells Hospital, Dundee, UK; $n = 100$). The study was conducted between March 2016 and July 2017 (total CAMEB study population, $n = 238$). All patients had radiologically confirmed bronchiectasis by high-resolution computed tomography scanning of the thorax interpreted in accordance with clinical practice guidelines of the British Thoracic Society (9). Patients were recruited during routine visits to the outpatient clinic, and were clinically stable at recruitment. Clinical stability was defined as the absence of new symptoms in patients and where no change had occurred to their bronchiectasis therapy in the preceding 6-week period. Patients were excluded if they had a primary diagnosis of any other major respiratory diagnosis (asthma or COPD, as defined by clinical symptoms and established spirometric criteria) (11, 12), were pregnant or breastfeeding, had active mycobacterial disease (identified through

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symptoms, chest radiograph, and sputum microbiology) (13), or were on chemotherapy for malignancy. Patients with any active infection requiring use of antibiotics or systemic corticosteroids in the 4 weeks preceding recruitment were also excluded. From the Singapore–Kuala Lumpur (SG-KL) cohort a total of 100 patients were matched individually by age, sex, and total bronchiectasis severity index (BSI) score (assigned at time of sample acquisition) to patients in the Dundee (DD) cohort (14). Patients with any prior history of or active ABPA (defined as meeting established International Society for Human and Animal Mycology criteria, including either type 1 *Aspergillus* skin test positivity or elevated IgE levels against *Aspergillus fumigatus* and elevated total IgE greater than 1,000 IU/ml, plus at least two of the following three criteria: precipitating or serum IgG antibodies against *A. fumigatus*; radiographic change consistent with ABPA; or total eosinophil count greater than 500 cells/μl in steroid-naive patients) at enrolment were excluded (15). A separate cohort of 149 patients with allergic rhinitis (AR), among which bronchiectasis was excluded by radiological examination, were recruited from the otolaryngology outpatient clinic at National University Hospital, Singapore, to serve as a comparator group with high allergic sensitization. AR was confirmed by evidence of sensitization to at least one allergen (by skin prick or serum IgE test) using established clinical criteria (16). The institutional review board of all the participating hospitals approved the study, and written informed consent was obtained from each patient.

Full details on clinical data and specimen collection, immunodot blot assay for specific IgE measurement, multiplex analysis for cytokine/chemokine quantification, as well as statistics and data analysis are provided in the supplementary materials.

Results

Clinical characteristics and patient demographics are shown in Table 1.

High Frequencies of Sensitization to a Range of Allergens Are Detected in Bronchiectasis

Based on prior findings from our group and others, we sought to comprehensively assess sensitization levels to multiple specific allergens in the CAMEB cohort (10, 17–20). Measured specific IgE (sIgE) titers against house dust mite (HDM), *Alternaria alternata* (Alt a), and recombinant allergens of *Aspergillus fumigatus* (rAsp) revealed high frequencies of sensitization in patients with bronchiectasis (Figure 1). Sensitization of class 3 or above (to at least one allergen) was observed in 57.6% ($n = 137$) of patients with bronchiectasis compared with 26.9% ($n = 40$) in the AR cohort, which served as an unmatched comparator group ($P < 0.0001$). Of all allergens tested, extracts of HDM (*Dermatophagoides pteronyssinus* [Der p] and *Blomia tropicalis* [Blo t]) elicited the highest median sIgE titers in bronchiectasis (Figure 1; *see also* Table E1 in the online supplement). Less than 3% ($n = 4$) of patients with AR exhibited sIgE titers in the "very high" (class 4 and above) range versus 33.2% ($n = 79$) in the bronchiectasis cohort. Only patients in the bronchiectasis group registered sIgE titers in the "very high" (class 6) range ($n = 35$; 14.7%).

Sensitization to Multiple Allergens Is Associated with More Severe Disease and Poorer Lung Function in Bronchiectasis

Having identified high levels of sensitization in bronchiectasis, we next investigated if this was associated with disease severity and poorer clinical progression, focusing on the primary clinical outcomes of BSI, lung function (FEV_1% predicted), and exacerbation rate in the prior year. Patients were categorized by the number of allergens to which each individual was sensitized (defined as sensitization of class 3 or greater). Higher sIgE titers were observed in those sensitized to two or more allergens. These individuals more frequently had titers in the very high (class 4–6) range (Figure 2, $P < 0.00001$). Patients with sensitization to three or more allergens had greatest disease severity and poorest lung function (Figures 2A and 2B), but no association was found with exacerbation frequency (Figure 2C).

Sensitization Patterns in Bronchiectasis Demonstrate Geographic Variation

In patients from both cohorts, SG-KL and DD, the association between sensitization and poorer lung function (Figure 2B) was statistically significant ($P < 0.01$), whereas the effect on BSI (Figure 2A) was more pronounced in the SG-KL cohort ($P < 0.01$) compared with the DD cohort ($P = 0.06$). Interestingly, we found distinct geographic allergen response profiles based on patient origin. Patients from the SG-KL cohort exhibited higher responses to HDM allergens compared with the DD cohort, of which the response to Der p was significant (Figure 3). In contrast, the DD cohort had greater responses to fungal allergens, including *A. alternata* and *A. fumigatus* (rAsp f 6, f 8, f 15, f 17) with one exception, the *A. fumigatus* major allergen, rAsp f 1, the response of which was significantly elevated in the SG-KL cohort (Figure 3). In specific matched patient analyses, the significant responses to Der p and rAsp f 1 remained in the SG-KL cohort, whereas the response to rAsp f 17 was the single relationship maintained in the DD cohort (Tables E2 and E3). The clinical consequence of varied geographic sensitization pattern did not necessarily correlate with their observed frequency: sensitization to HDM allergens and rAsp f 1 was associated with poorer lung function in SG-KL and DD cohorts, respectively, whereas sensitization to rAsp f 17 was only linked to a higher exacerbation frequency in patients from the SG-KL cohort, despite its predominance in matched patients from the DD cohort (Table E4).

Sensitization to the *A. fumigatus* Minor Allergen rAsp f 17 Is Enriched in Bronchiectasis-associated Serological ABPA

All recruited patients with bronchiectasis were managed in accordance with established European Respiratory Society guidelines that advise screening for ABPA at diagnosis: we excluded all patients that screened positive in accordance with our study criteria, but, in some cases, this screening would have been several years prior (2). Despite this strict exclusion criteria, when all *Aspergillus*-sensitized patients in our bronchiectasis cohort were considered ($n = 224$; 95.8%), 43 (18.1%) met the criteria for serological ABPA (s-ABPA) based on the published classification used in CF-related bronchiectasis (21).

In these patients, we next assessed the relationship between the presence or absence of s-ABPA, and sensitization to specific *A. fumigatus* allergens. This revealed a significant association between s-ABPA and an rAsp f 17–specific response, a relationship statistically driven by differences in the DD cohort (Figures 4A

Table 1. Demographics Table Illustrating Patient Cohorts of Allergic Rhinitis ($n = 149$) Compared with the Cohort of Asian and Matched European Bronchiectasis of Non–Cystic Fibrosis Bronchiectasis ($n = 238$)

Characteristic	Allergic Rhinitis (*n = 149*)	Patients with Bronchiectasis (*n = 238*)	Patients with Bronchiectasis SG-KL (*n = 138*)	Matched Cohorts		
				SG-KL (*n = 100*)	DD (*n = 100*)	*P* Value
Age, yr, median (IQR)	28 (23–35)	68 (64–71)	65 (58–73)	65 (58–74)	69 (64–76)	0.021
Sex, *n* (%)						0.392
Female	60 (40)	130 (55)	77 (55)	59 (59)	53 (53)	
Male	89 (60)	108 (45)	61 (45)	41 (41)	47 (47)	
Etiology, *n* (%)						0.040
Idiopathic	—	145 (61)	85 (62)	63 (63)	60 (60)	
Postinfection (nonmycobacterial)	—	51 (21)	25 (18)	18 (18)	26 (26)	
Postinfection (mycobacterial)	—	19 (8)	18 (13)	9 (9)	1 (1)	
Other	—	23 (10)	10 (7)	10 (10)	13 (13)	
Smoking status, *n* (%)						0.013
Never	—	170 (70)	108 (78)	80 (80)	62 (62)	
Current	—	11 (5)	7 (5)	4 (4)	4 (4)	
Past	—	57 (25)	23 (17)	16 (16)	34 (34)	
BSI status, *n* (%)						0.355
Severe	—	147 (62)	84 (61)	63 (63)	63 (63)	
Moderate	—	71 (30)	45 (33)	26 (26)	26 (26)	
Mild	—	20 (8)	9 (6)	11 (11)	11 (11)	
BSI score, median (IQR)	—	9 (6–13)	10 (7–14)	10 (7–14)	9 (6–12)	0.054
BMI, kg/m^2, median (IQR)	—	21 (18–27)	19 (17–22)	19 (17–22)	27 (22–31)	<0.001
MRC dyspnea score, *n* (%)						
1–3	—	200 (84)	121 (88)	90 (90)	79 (79)	0.001
4	—	26 (11)	10 (7)	6 (6)	16 (16)	
5	—	12 (5)	7 (5)	4 (4)	5 (5)	
FEV_1% predicted, median (IQR)	—	74 (54–87)	69 (51–84)	69 (52–84)	76 (57–96)	0.067
Radiological severity, *n* (%)						0.123
1–2 lobes involved	—	106 (45)	62 (45)	43 (43)	44 (44)	
3 or more lobes involved	—	132 (55)	76 (55)	57 (57)	56 (56)	
No. of exacerbations in previous year, *n* (%)						<0.001
0	—	84 (35)	69 (50)	44 (44)	15 (15)	
1–2	—	82 (35)	51 (37)	41 (41)	31 (31)	
3 or more	—	72 (30)	18 (13)	15 (15)	54 (54)	
Hospital admissions before study, *n* (%)						0.004
Yes	—	88 (37)	63 (46)	43 (43)	25 (25)	
No	—	150 (63)	75 (54)	57 (57)	75 (75)	
Colonization with other organisms, *n* (%)						0.002
Yes	—	127 (53)	60 (43)	44 (44)	67 (67)	
No	—	111 (47)	78 (57)	56 (56)	33 (33)	
Pseudomonas colonization, *n* (%)						0.032
Yes	—	23 (10)	18 (13)	15 (15)	5 (5)	
No	—	215 (90)	120 (87)	85 (85)	95 (95)	
Bronchodilator use, *n* (%)						0.158
Yes	—	107 (45)	58 (42)	39 (39)	49 (49)	
No	—	131 (55)	80 (58)	61 (61)	51 (51)	
Inhaled corticosteroids, *n* (%)						<0.001
Yes	—	80 (34)	21 (15)	14 (14)	59 (59)	
No	—	158 (66)	117 (85)	86 (86)	41 (41)	
Mucolytics, *n* (%)						<0.001
Yes	—	118 (50)	60 (44)	45 (45)	13 (13)	
No	—	120 (50)	78 (56)	55 (55)	87 (87)	
Long-term antibiotics, *n* (%)						0.032
Yes	—	48 (20)	22 (16)	14 (14)	26 (26)	
No	—	190 (80)	116 (84)	86 (86)	74 (74)	

Definition of abbreviations: BMI = body mass index; BSI = bronchiectasis severity index; CF = cystic fibrosis; DD = Dundee; IQR = interquartile range; MRC = Medical Research Council; SG-KL = Singapore–Kuala Lumpur.

Demographics table illustrating the patient cohorts with allergic rhinitis ($n = 149$) and non-CF bronchiectasis ($n = 238$). The non–CF bronchiectasis cohort includes a matched cohort of patients from SG-KL and DD. Matching was performed based on age, sex, and disease severity (total score on the BSI). The variables defining the composite BSI score include BMI, shortness of breath (MRC) dyspnea score, FEV_1% predicted values, radiological severity by number of involved lobes, number of exacerbations and hospitalizations in the preceding year, microbial colonization with other organisms, and colonization by *Pseudomonas aeruginosa*. Relevant therapy is documented, including bronchodilator, inhaled corticosteroid, and mucolytic or long-term prophylactic antibiotic use. Data are presented as median (IQR) or *n* (%), and *P* values for differences observed between matched cohorts are indicated in the rightmost column.

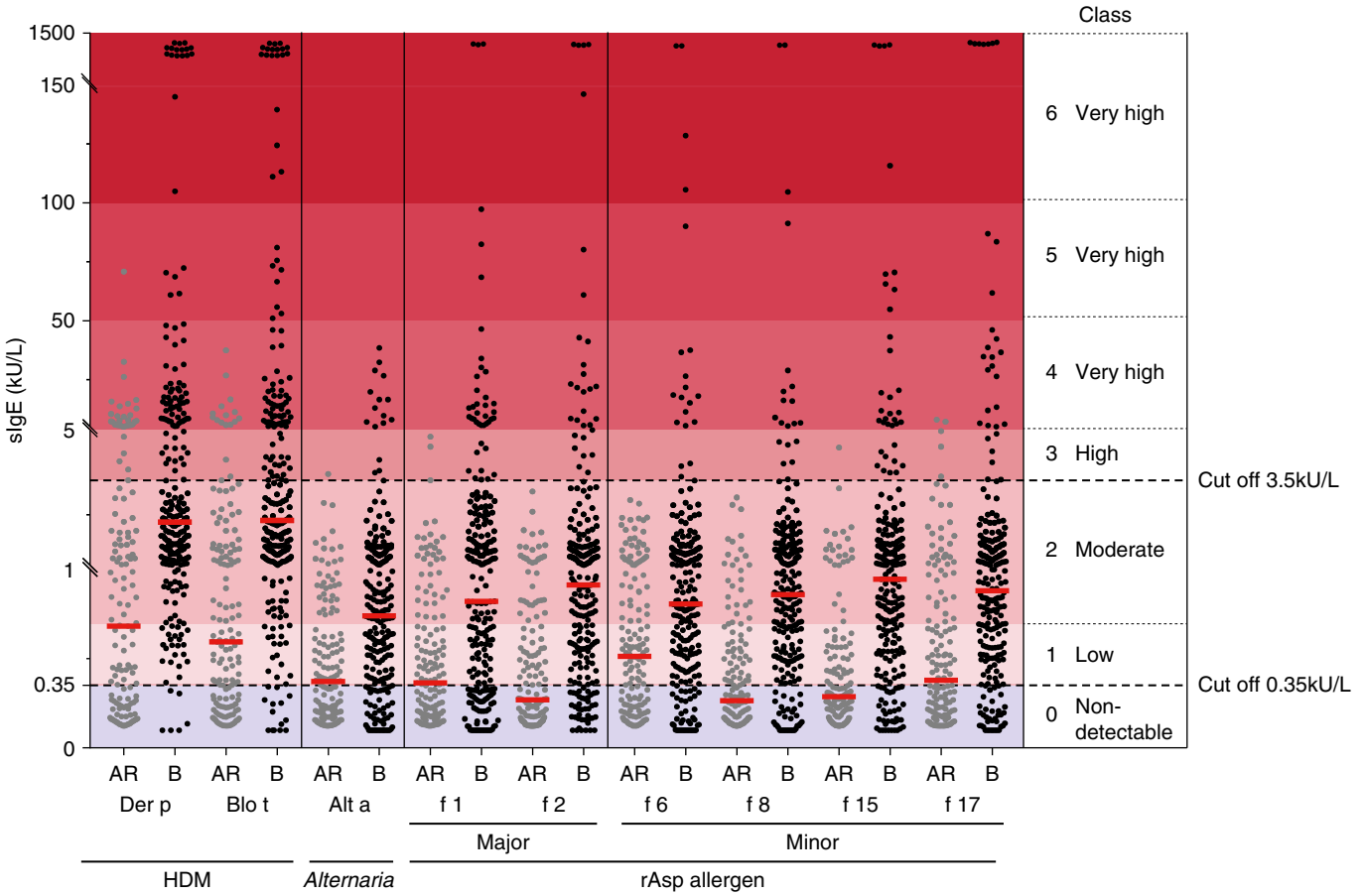

Figure 1. High frequencies of sensitization to house dust mite (HDM), *Alternaria alternata* (Alt a), and recombinant allergens of *Aspergillus fumigatus* (rAsp) are detected in stable non–cystic fibrosis bronchiectasis. Studied allergens are denoted as follows; HDM (*Dermatophagoides pteronyssinus* [Der p], *Blomia tropicalis* [Blo t]), *A. alternata* (Alt a), and rAsp (f 1, f 2 [Major allergens] and f 6, f 8, f 15, f 17 [minor allergens]). Specific IgE (sIgE) titers (as kU/L) against each allergen are indicated: bronchiectasis (B; black) and allergic rhinitis (AR; gray). Median values for all groups are illustrated (red lines).

and 4B). Importantly, however, a trend toward enrichment of a sensitization response to rAsp f 17 was also observed in the SG-KL cohort (Figure 4B). When all patients with s-ABPA are considered (from both SG-KL and DD cohorts), a significantly higher proportion have sensitization responses to rAsp f 17 in the class 3 or above range (Table E4). Those with rAsp f17 sensitivity also had higher rates of quantitative PCR positivity for *A. fumigatus* (69% vs. 45%, $P = 0.017$), whereas their sputum galactomannan levels were comparable (65% vs. 69%, $P = 0.801$), measures determined in a previously reported analysis of the CAMEB cohort (10). Interestingly, although the highest observed responses to rAsp f 17 were in Scottish patients with s-ABPA, Singaporean and Malaysians who had significant responses to rAsp f 17 also had significantly more exacerbations (median = 1.5 vs. 0; $P < 0.05$), suggestive that responses to this allergen are clinically relevant in both populations (Figure 4, Table E4).

Clinically Relevant "Immunoallertypes" Are Defined by Sensitization Pattern and Airway Immune Profiling in Bronchiectasis

We next investigated whether clinically relevant patient clusters based on sensitization pattern and airway immune profiles (immunoallertypes) occur in bronchiectasis. To address this, we used the multiplex sputum cytokine and chemokine profiling approach, previously validated in COPD, to assess immunological signals, and linked this to sensitization patterns and accompanying clinical phenotypes (22). Hierarchical cluster analysis of resultant data revealed two distinct patient immunoallertypes characterized by distinct sensitization patterns and immune profile (Figure 5A). Assignment into immunoallertypes was not driven by geographic origin or the presence of s-ABPA, as, within each group, we found equal proportions of patients from both SG-KL and DD cohorts, as well as an equal distribution of s-ABPA ($P > 0.05$; Figure 5A). Each immunoallertype was instead defined by a unique sensitization pattern and immune profile: patients in the fungal-driven proinflammatory (FDPI) group exhibited marked responses to the fungal allergens Alt a and rAsp coupled to a proinflammatory profile characterized by elevated airway TNF-α, IL-1α, and IL-1β. Asthma-like symptoms, long-term antibiotic use ($P = 0.0620$), or increased inhaled corticosteroid use ($P = 0.932$) was not significantly apparent in either immunoallertype (Table E5), nor was inhaled corticosteroid use significantly different in those exhibiting sensitization

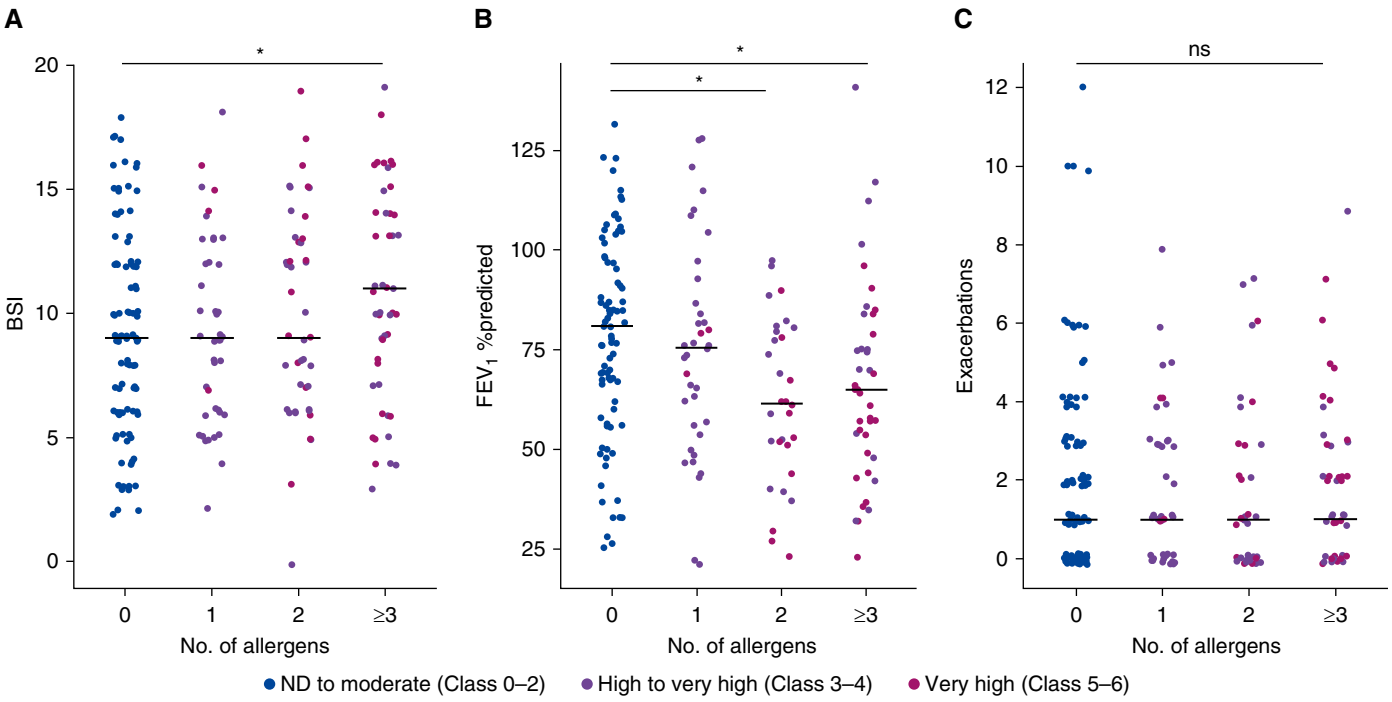

Figure 2. Sensitization to multiple (≥3) allergens in non–cystic fibrosis bronchiectasis is associated with more severe disease and poorer pulmonary function but does not affect exacerbations. The number of allergens to which an individual is sensitized (defined as specific IgE [sIgE] class ≥3) was examined in relation to (*A*) disease severity (as bronchiectasis severity index [BSI]), (*B*) pulmonary function (as FEV_1% predicted), and (*C*) exacerbation frequency in the preceding year. Dot coloration indicates sIgE class range as: nondiseased to moderate sensitization (class 0–2; blue); high to very high (class 3–4; purple); and very high (class 5–6; pink). For patients exhibiting sensitization to more than a single allergen, the coloration corresponding with the highest sIgE class range is illustrated. Median values for all groups are shown (black lines). ND = nondiseased; ns = not significant. *$P \leq 0.05$.

(sIgE class 3 or greater, $P = 0.580$). However, higher levels of sputum galactomannan, trending toward significance, were observed in patients with FDPI and bronchiectasis ($P = 0.065$, Table E5). In contrast, patients in the HDM-driven, chemokine-dominant (HDCD) group exhibited lower sensitization to fungal allergens, but significant responses to HDM allergens, that was accompanied by a chemokine-dominant airway profile characterized by high GRO (growth-regulated oncogene) (CXCL1), MCP-1 (monocyte chemoattractant protein-1) (CCL2), and eotaxin-1 (CCL11). This HDCD immunoallertype also exhibited anti-inflammatory signatures, including elevated IL-1RA, IL-10, and G-CSF (granulocyte colony–stimulating factor) (Figure 5A). After patient stratification by underlying immunoallertype, a significant association was observed in the FDPI pattern with bronchiectasis severity. The FDPI immunoallertype conferred significantly worse disease (Figure 5B, Table E5) and poorer lung function (Figure 5C, Table E5), whereas frequent exacerbators were equally observed in both groups (Figure 5D). Postinfective bronchiectasis and greater long-term antibiotic use was also apparent in the FDPI group (Table E5).

Geographic Patient Origin Drives Intraimmunoallertype Variation in Bronchiectasis

Given our observed geographic differences in sensitization pattern in bronchiectasis (Figure 3, Tables E2 and E3), we next assessed if there was intraimmunoallertype variation, based on patient geographic origin. Our previously defined immunoallertypes were based on unsupervised hierarchical clustering and categorized patients into two groups, with equal frequencies of patients from each of our geographic cohorts (SG-KL and DD; Figure 5A, ii). To investigate intraimmunoallertype patient variation based on geographic origin, we used a supervised Markov blanket approach to identify features within each immunoallertype associated with SG-KL and DD, respectively. As such, intraimmunoallertype variability was assessed in terms of information shared between sensitization pattern, immune cytokine/chemokine profile, and geographic patient origin (Figure 6). In the FDPI group, Asian origin (SG-KL cohort) was associated with a predictive set of features, including IFN-α2, TNF-β, and the PDGF (platelet-derived growth factor) isoforms AA and AB/BB, whereas European origin (DD cohort) correlated with greater levels of sCD40L, IL-1RA, and IL-17A (Figure 6A). In the HDCD group, patients from the SG-KL cohort exhibited an increased MCP-3, IL-1β, and PDGF-AB/BB, and a significant HDM allergen response, whereas IL-9, IL-10, TGF-α (transforming growth factor-α), MDC (macrophage-derived chemokine), eotaxin, and FGF-2 (fibroblast growth factor-2) were more predictive of DD cohort membership (Figure 6B). This suggests the existence of subgroups based on geographic origin within each of the defined immunoallertypes and indicates the potential clinical importance of geographic heterogeneity in bronchiectasis. The strongly associated HDM response identified in the SG-KL cohort (Figure 6B) is consistent with our earlier analyses (Figure 3), which lends further credence that immunoallertypes are clinically relevant. Independent of immunoallertype, the SG-KL

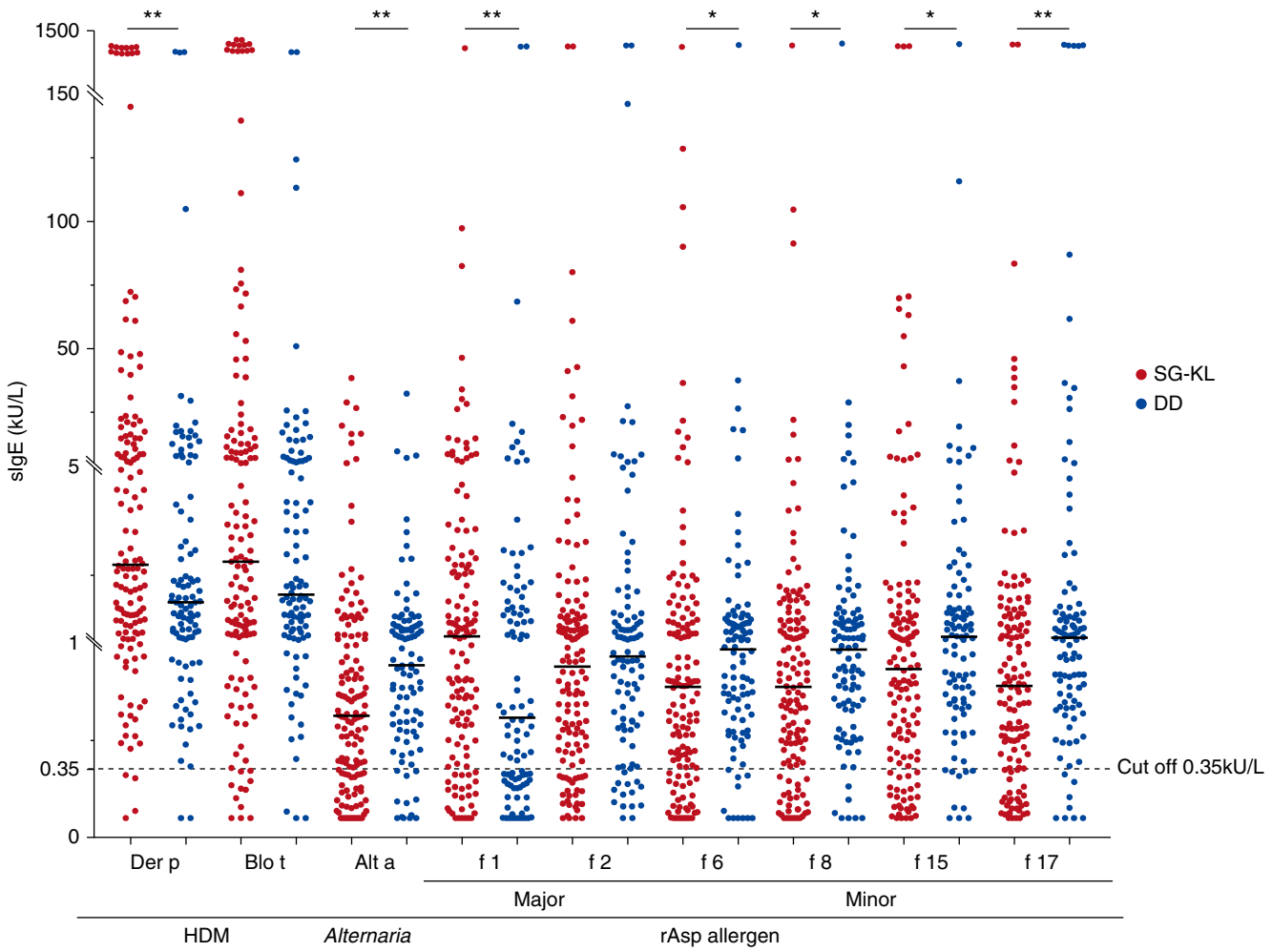

Figure 3. Geographic variation in sensitization profiles to specific recombinant allergens are observed in non–cystic fibrosis bronchiectasis. Patients from the Singapore–Kuala Lumpur (SG-KL) cohort (red dots) exhibit significantly higher specific IgE (sIgE) titers to *Dermatophagoides pteronyssinus* (Der p) and the major *Aspergillus fumigatus* allergen rAsp f 1, whereas patients from the Dundee (DD) cohort (blue dots) have higher responses to *Alternaria alternata* (Alt a) and all minor *Aspergillus* allergens (rAsp: f 6, f 8, f 15, and f 17). Median values for all groups are shown (black lines). rAsp f = recombinant *A. fumigatus* allergen. $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. HDM = house dust mite.

and DD cohorts further illustrate significant differences in airway inflammatory signature, for example, elevated PDGF-AB/BB ($P < 0.0001$) and MCP-3 ($P < 0.05$) in Singaporean and Malaysian patients, substantiating regional differences in immune response. Eotaxin is significantly elevated in sensitized bronchiectasis ($P < 0.05$), but no differences in inflammatory patterns are observed between patients with bronchiectasis with different underlying etiologies (e.g., idiopathic, postinfectious, or other causes).

Discussion

Atopy and sensitization are important "treatable traits" in asthma, COPD, and their associated overlap syndromes, but their role in bronchiectasis remains uncertain (5, 17, 23). The present study is the most comprehensive to date addressing this, and detected high frequencies of sensitization to a range of specific allergens, exceeding that found in a comparator AR cohort, which although unmatched, served as an important positive control group of heightened sensitization. Sensitization has clinical implications in bronchiectasis and demonstrates geographic variation in allergen response pattern. Individual allergen responses correlate with specific clinical outcomes in bronchiectasis, including FEV_1 (HDM and rAsp f 1), exacerbations (rAsp f 17), and the presence of s-ABPA (rAsp f 17). Two clinically relevant immunoallertypes are described—FDPI and HDCD—with the FDPI pattern linked to poorer clinical outcome.

Sensitization induced by HDM is common, and may associate with poor clinical outcomes in allergic respiratory disease (4, 24). The effect of HDM exposure in asthma, for instance, relates to a T-helper cell type 2 (Th2)–mediated, chemokine-associated allergic response of deleterious clinical consequence (24, 25). In COPD, increased sensitization is implicated in disease pathogenesis and correlates with exacerbations (5). In our work, significant numbers of apparently stable patients with bronchiectasis show HDM sensitization, which presumably reflects either coexisting subclinical allergic airways disease or a

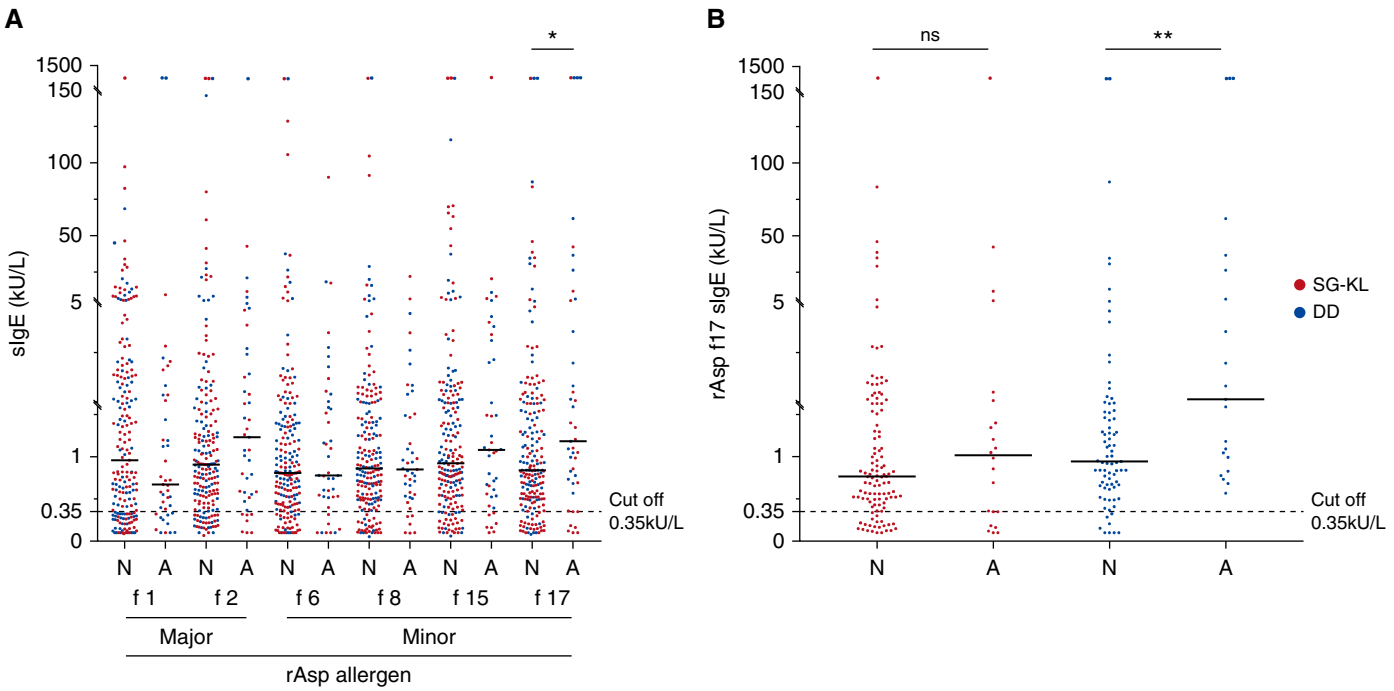

Figure 4. Sensitization to rAsp f 17 is enriched in serologic allergic bronchopulmonary aspergillosis (s-ABPA). (*A*) Specific IgE (sIgE) titers against recombinant *Aspergillus fumigatus* allergens (major, f 1 and f 2; minor, f 6, f 8, f 15, and f 17) in the absence or presence of s-ABPA (N and A, respectively) illustrates enrichment for significant responses to rAsp f 17 in s-ABPA and (*B*) significant sensitization to rAsp f 17 is observed in the Dundee (DD) (blue dots) but not Singapore–Kuala Lumpur (SG-KL) (red dots) cohorts with s-ABPA. Median values for all groups are shown (black lines). ns = not significant; rAsp f = recombinant *A. fumigatus* allergen. $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$.

predisposition to atopy after airway damage caused by bronchiectasis. Given the dearth of bronchiectasis therapy currently available, and the known implications of allergic sensitization on clinical outcomes in other respiratory diseases, addressing HDM sensitization in bronchiectasis offers an important therapeutic avenue. The role of fungal allergy in respiratory disease is well recognized, and fungal sensitization is an established prognostic indicator in severe asthma with fungal sensitization and ABPA, and has also been reported in COPD, where specific fungal responses are implicated as a risk factor for progression to bronchiectasis (4, 17, 23). This latter work supports our findings of high rates of atopy and sensitization in bronchiectasis, highlighting a potential role for *A. fumigatus* even in non-ABPA respiratory disease (6, 17). Our comprehensive immune-allergy assessment of stable patients with bronchiectasis importantly identified patients with s-ABPA; a potentially treatable condition, which would have been missed by guideline-recommended screening alone, particularly as the s-ABPA developed after the initial diagnosis of bronchiectasis.

Allergen-based sensitization patterns exhibit regional and global variability, and marked differences have been observed across Asian and European populations (4, 24, 26). Our group has previously reported a high prevalence of HDM sensitization in the Asian setting, a feature confirmed in bronchiectasis (23, 26). Allergen exposure varies by country and with different ethnic backgrounds, and, as such, a key strength of our study is in its multicenter design. Geographically distinct allergen profiles were observed across our matched cohorts, not just to HDM, but also to *A. alternaria* and specific *A. fumigatus* allergens. To our knowledge, this is the first study to compare such populations in bronchiectasis. Singapore and Malaysia (where our Asian patients were recruited) have tropical climates and distinct allergen profiles, which, when coupled to genetic and other environmental differences, including air quality and use of air conditioning, may potentially explain the observed differences compared with temperate oceanic climates seen in Scotland (where our European patients were recruited) (4, 24). Differences in sensitization between populations (for instance, to the same *A. fumigatus*–specific allergen) may be due to host genetic and immunological variation, but allergen cross-reactivity with other fungi may also be important, given the potential contrasting environmental exposures. Further, Blo t sIgE responses were observed among the European population, despite the absence of this tropical dust mite species in DD. We suspect that this is due to the recognition of cross-reactive epitopes of Der p or other HDM species, rather than those of Blo t. Critically, our observed allergen-associated patterns relate differently to clinical outcomes in the two regions: HDM and rAsp f 1 responses relate to FEV_1 in Asians and Europeans, respectively, whereas increased exacerbations were linked to the rAsp f 17 response in Asians and to s-ABPA in both populations. An improved understanding of the role of environmental exposures and its geographic variation will ultimately require a more direct environmental measurement of patient exposure, preferably at the point of sampling, to fully explicate the precise influence a patient's environment may have on sensitization and disease progression in bronchiectasis.

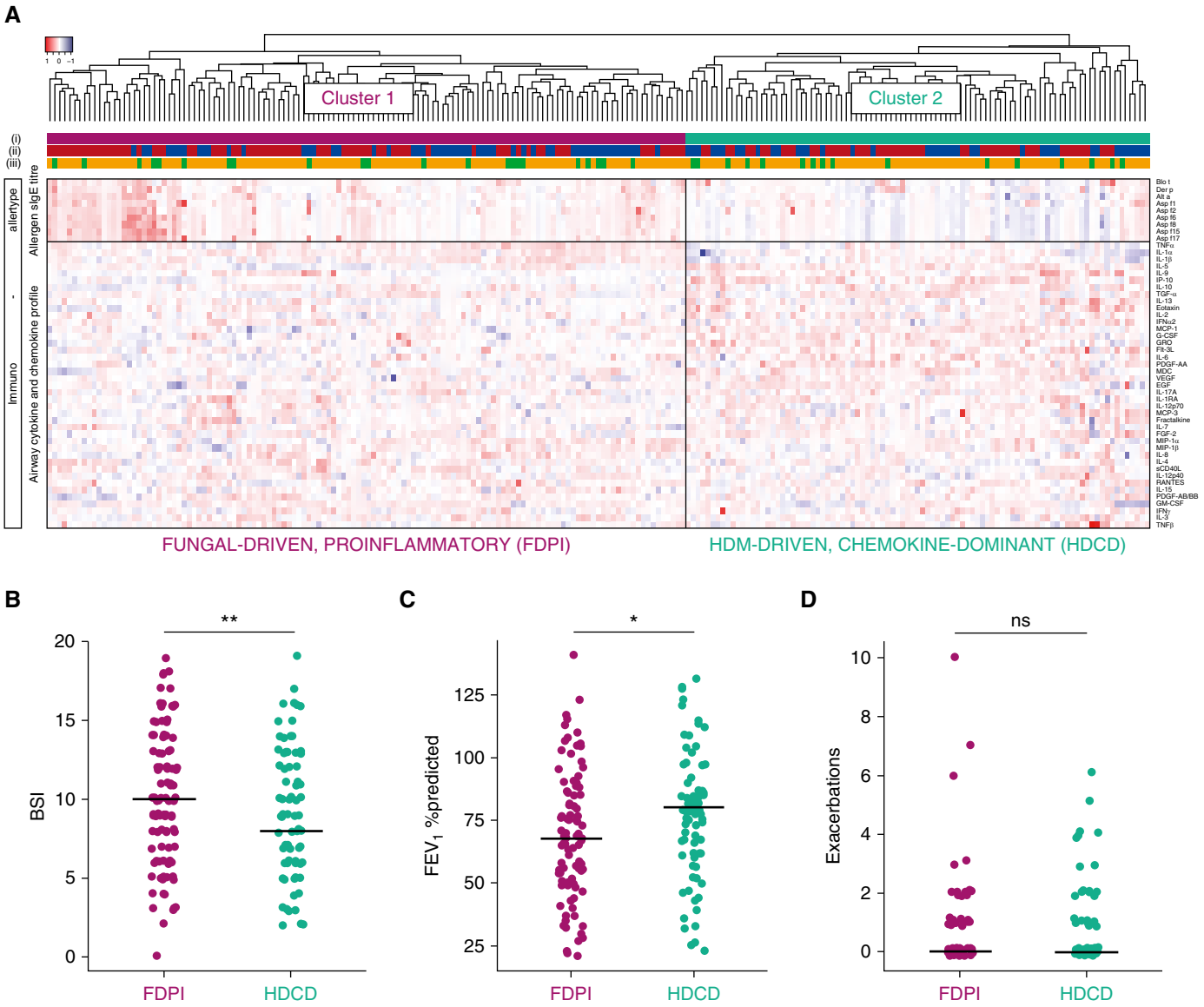

Figure 5. Pattern of allergen sensitization and associated airway inflammatory state defines clinically relevant immunological clusters of allergy in non–cystic fibrosis bronchiectasis ("immunoallertypes"). (*A*) Hierarchical cluster analysis based on systemic specific IgE (sIgE) titer against a range of allergens and airway cytokine/chemokine expression reveals two distinct clusters ("immunoallertypes") in bronchiectasis: fungal driven proinflammatory (FDPI; cluster 1) and house dust mite (HDM) driven, chemokine dominant (HDCD; cluster 2). A dendrogram illustrating hierarchical clustering of patients with bronchiectasis and their associated immune profiles (by heat map) are illustrated. Colored bars (below dendrogram) denote (i) cluster membership (cluster 1, purple; cluster 2, turquoise); (ii) geographic patient origin (Singapore–Kuala Lumpur, red; Dundee, blue); and (iii) serologic allergic bronchopulmonary aspergillosis (s-ABPA) status (non-ABPA, orange; s-ABPA, green). Membership in cluster 1 is associated with (*B*) increased disease severity (based on bronchiectasis severity index [BSI]) and (*C*) poorer pulmonary function (as FEV_1% predicted); however, (*D*) no difference in exacerbation frequency was detected between clusters. ns = not significant. $^*P \leq 0.05$ and $^{**}P \leq 0.01$.

A response to rAsp f 17 is of particular importance in bronchiectasis. This allergen, encoded by the Afu4 g03240 gene of the *A. fumigatus* AF293 genome, was originally characterized by Yuen and colleagues (27) as the first species-specific antigenic cell wall galactomannoprotein, but its clinical relevance outside invasive aspergillosis is unclear. Our study, therefore, also represents the first clinical evidence of a potential role for this allergen in chronic airways disease, and specifically in bronchiectasis, where it is linked to exacerbations and s-ABPA. Interestingly, Gibbons and colleagues (28) demonstrate that rAsp f 17 exhibits significant upregulation (>200-fold) in *A. fumigatus* biofilms, much more than any other specific allergen. Taken together, this links rAsp f 17 sensitization with *A. fumigatus* biofilm production and matrix galactomannan concentration. Therefore, further work is now warranted to better understand its role in immunopathogenesis and potential clinical use in *Aspergillus*-associated allergic disease.

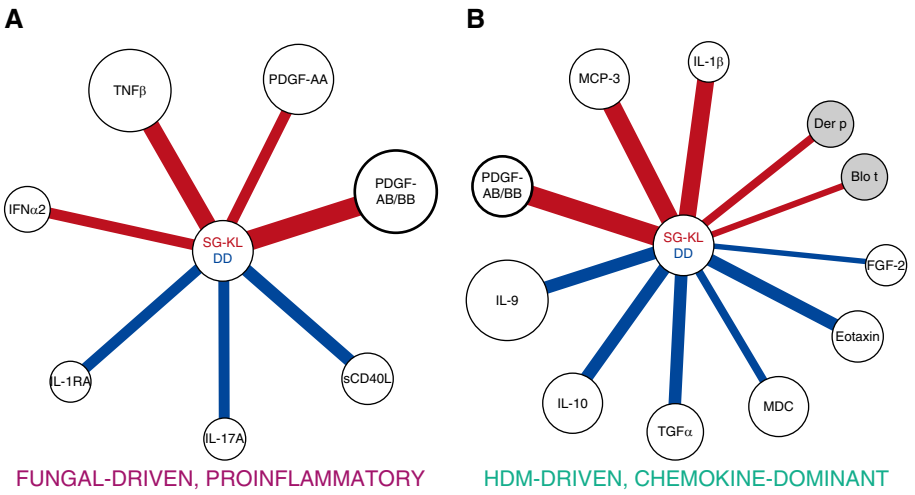

Figure 6. Geographic origin associates with intracluster variation in immune profiles that distinguish patients with bronchiectasis of Asian and European origin. Mutual information networks illustrating associations between immune profile and geographic patient origin are shown for (*A*) cluster 1 (fungal driven proinflammatory) and (*B*) cluster 2 (house dust mite [HDM] driven, chemokine dominant). A central circle represents the target node of geographic patient origin (Singapore–Kuala Lumpur [SG-KL], red; Dundee [DD], blue), and positively correlated immune profiles (by Pearson correlation) are represented by outer circles (sized to reflect the mutual information shared with the target node). Line thickness illustrates the strength of the correlation, and line coloration indicates geographic origin: SG-KL (red) and DD (blue), respectively. Cytokines and chemokines (white outer circles) and allergens (gray outer circles) are shown. Immune analytes common to both networks are further highlighted by a thickened border. Blo t = *Blomia tropicalis*; Der p = *Dermatophagoides pteronyssinus*; FGF-2 = fibroblast growth factor-2; MCP-3 = monocyte chemotactic protein-3; MDC = macrophage-derived chemokine; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TNF = tumor necrosis factor.

When sensitization pattern and airway immune response are considered together in unsupervised analyses, two distinct immunoallergic signatures of clinical relevance emerge. The first of these groups—FDPI—is fungal driven, characterized by specific responses to rAsp allergens and an airway rich in the proinflammatory cytokines, IL-1α, IL-1β, and TNF-α. These cytokines trigger release of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 from the endothelium leading to neutrophil and eosinophil airway recruitment, and their expression in the FDPI group is notable, given the central importance of neutrophils in bronchiectasis (29–32). After cell recruitment, increased airway smooth muscle contractility and hyperresponsiveness may result, potentially explaining the poorer lung function and increased bronchiectasis severity in this group. This contrasts the HDM-associated (HDCD) immunoallertype identified by specific responses to Der p and Blo t and an airway rich in chemokines (eotaxin and IP-10 [IFN-γ-induced protein-10]), lymphokines (IL-9 and IL-2), growth factors (TGF-α) and elevated concentrations of Th2-related cytokines (IL-5, IL-10, and IL-13) consistent with Th2-dominant airway inflammation, suggestive of eosinophilic involvement. Taken together, these identified endophenotypes of bronchiectasis may be amenable to targeted treatments, including anti-inflammatories, corticosteroids, anti–Th2-cytokine, or antifungal therapy, a strategy that has shown positive outcomes for patients with CF colonized by *A. fumigatus* (33). Frequent exacerbators were seen in both immunoallertypes with equal frequency, suggesting that this important phenotypic endpoint used in clinical trials of bronchiectasis therapies may be underpinned by alternate mechanisms, necessitating more targeted approaches. Though the idea that different immunological pathways could drive sensitization and exacerbations in bronchiectasis is intriguing, we recognize that our study is limited by its size. Smaller subgroups associated with exacerbations may exist, which our study lacked power to detect. Notwithstanding this, the fact that exacerbation rates were equivalent among the different immunoallertypes serves to highlight that potential "exacerbation types" may exist in bronchiectasis, analogous to observations in COPD and asthma, where potentially treatable traits include specific endotype-driven exacerbations (1, 34–36). Moreover, although exacerbation remains a key bronchiectasis phenotype, its use as an endpoint in clinical trials for bronchiectasis has proven challenging (37, 38). Treatable traits, such as sensitized bronchiectasis, do not themselves represent clinical endpoints; however, identifying such endophenotypes amenable to focused therapy addresses other important clinical symptoms in bronchiectasis (1, 39–41). Our distinct immunoallertypes, when assessed together with bronchiectasis symptomology, may allow better patient stratification, which in turn permits tailored, focused, and effective interventions for this highly heterogeneous disease.

A limitation of this work is its cross-sectional nature, precluding assessment of the temporal dynamics of the identified immunoallertypes, their risk factors, and underlying causal mechanisms. Our ability to assess cause–effect relationships between sensitization and clinical outcome was largely constrained by our decision to focus on matching our patient cohorts on age, sex, and disease severity, which, by nature, creates logistical challenges for longitudinal sampling. Longitudinal studies in bronchiectasis are nonetheless an important and on-going research focus of our group and others, as we seek to explicate further the clinical correlates of immunoallertype profiles as well as the therapeutic factors, such as long-term macrolide therapy, which may influence the immunoallertype itself through immunomodulation. Such effects were likely undetected in this study due to its cross-sectional design. Furthermore, we did not perform analyses in other comparator pulmonary cohorts, such as asthma, or assess sputum immune cells, which would have further substantiated the observed immunoallertypes, and, although we identified an increasing trend in galactomannan sputum positivity in patients with FDPI, we did not investigate corresponding airway markers in the HDCD group, such as dust mite protease. Though our allergen panel was broad, it can be further extended to include cockroach, animal dander, or geographically prominent pollens—all of which are areas of future work.

Bayesian network analysis revealed specific immune profiles among our geographically distinct cohorts, suggesting regional variability within immunoallertypes

with implications for endophenotyping in bronchiectasis. In the SG-KL cohort, the FDPI immunoallertype had associated features linked to an airflow-limiting airway remodeling process, notably PDGF and TNF-β expression. In contrast, patients from the DD cohort exhibiting the FDPI immunoallertype had distinctive immune signatures, comparable to those observed in allergic asthma implicating IL-RA and IL-17A (42–44). Both geographic groups classed as HDCD demonstrated a chemokine-dominant, proinflammatory milieu with increased growth factor expression, reflective of a fibrotic airway remodeling process. The implication of PDGF-AB/BB is of particular interest, given the recently described hematopoietic potential of the lung and reported links between exacerbation and platelets in COPD (45, 46). Though interesting, replication of these geographic differences in larger cohorts is required before true clinical importance can be established. Perhaps more intriguing was the degree of similarity seen across such geographically distinct populations, serving to broadly validate the observed immunoallertypes and their association with clinical outcome in bronchiectasis.

The interplay between allergens, sensitization, and the immune-inflammatory response in bronchiectasis is complex with apparent geographical variation. Our work reveals, for the first time, high frequencies and distinct patterns of sensitization in bronchiectasis. Although therapies employed in other allergic respiratory diseases may now be considered in patients with bronchiectasis complicated by allergic sensitization, we must address the significant and inherent heterogeneity in this disease. The immunoallertypes presented here represent an important starting point of future work focusing on improved patient endophenotyping in bronchiectasis, which, in turn, will allow stratified therapeutic approaches to sensitized bronchiectasis: a key treatable trait. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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